REMARKS

Applicants have amended their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have amended claim 8 to delete recitation, from the preamble, that the genes are derived from different samples, and to recite that the first sample is derived from a first specimen, and that the second sample is derived from a second specimen; claim 8 has been further amended to recite that a sequence of the targeted gene included in the first nucleotides and a sequence of the targeted gene included in the second nucleotides are the same. Moreover, Applicants have cancelled claim 12 without prejudice or disclaimer.

In addition, Applicants are adding new claims 13-15 to the application. Of these newly added claims, claim 14 is an independent claim, reciting a method for gene expression analysis. Claim 14 is similar to claim 8 as presently amended, but recites that the first sample is derived from a first tissue or organ, and that the second sample is derived from a second tissue or organ. Claims 13 and 15, dependent respectively on claims 8 and 14, recite that the second specimen (tissue or organ) is a different specimen (tissue or organ) than the first specimen (tissue or organ).

Applicants respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims in the Office Action mailed May 16, 2006, that is, the teachings of the U.S. Patent to Shah, et al., No. 6,165,723, and International (PCT) Publication No. WO 97/42345 (Whitcombe, et al.), under the provisions of 35 USC 102 and 35 USC 103.

It is respectfully submitted that the references as applied by the Examiner, either alone or in combination, would have neither taught nor would have suggested such a method for gene expression analysis as in the present claims, including, inter alia, preparing first nucleotides including a targeted gene by using a first sample derived from a first specimen (tissue or organ), and preparing second nucleotides including this targeted gene by using a second sample derived from a second specimen (tissue or organ) the preparation of the first and second nucleotides including introducing various base sequences which are nonspecific to the base sequence of the targeted gene, to the targeted gene so that the various base sequences are bound at recited positions relative to the 5' end; mixing the first and second nucleotides; and subjecting the first and second nucleotides to nucleic acid amplification using various primers and probes, and thermostable DNA polymerase having specified activity, with digesting of the first and second probes and detecting a fluorescence emitted by respective fluorophores released in digesting the first and second probes, to thereby assay the amount of the product of the nucleic acid amplification, and wherein a sequence of the targeted gene in the first and second nucleotides is the same. Note claim 8. See also claim 14.

Furthermore, it is respectfully submitted that these references as applied by the Examiner would have neither taught nor would have suggested such method for gene expression analysis as in the present claims, having features as discussed previously in connection with claims 8 and 14, and, moreover, wherein the second specimen tissue or organ is a different specimen tissue or organ from the first specimen tissue or organ. See claims 13 and 15.

Thus, the method according to the present invention includes expression analysis of two genes having different sequences derived from different specimens

or tissues/organs. The method according to the present invention is intended to be used, for example, for simultaneous analysis of similar genes having different sequences derived from several different samples (for example, as in Example 2 starting from page 19 of Applicants' specification, GAPDH derived from a mouse liver and GAPDH derived from a mouse kidney).

According to the method of the present invention, first nucleotides prepared from a first sample derived from a first specimen (tissue/organ), and second nucleotides prepared from a second sample derived from a second specimen (tissue/organ), are subjected to nucleic acid amplification using a specified first probe including a base sequence identical or complementary to a first base sequence used in preparing the first nucleotides, and labeled at one end with a first fluorophore and at another end with a quencher, and a specified second probe including a base sequence identical or complementary to a third base sequence used in preparing the second nucleotides, and labeled at one end with a second fluorophore and at another end with a quencher, with fluorescence emitted by the first and second fluorophores released in digesting the first and second probes being detected in digesting the probes. In this method, a sequence of the targeted gene included in the first nucleotides and a sequence of the targeted gene included in the second nucleotides are the same. By this method, highly accurate quantitative analysis of expression levels of the targeted gene in first and second samples (e.g., different samples, derived from different specimens or tissues/organs) by real-time PCR (polymerase chain reaction) detection under substantially the same conditions can be achieved. Note, for example, the second paragraph on page 15, and the first paragraph on page 22, of Applicants' specification.

In addition, it is respectfully submitted that the teachings of the applied references would have neither disclosed nor would have suggested such method for gene expression analysis as in the present claims, having features as discussed previously in connection with claim 8, and, additionally, the further definition of synthesizing the first and second nucleotides as recited in claim 9; and/or the further definition of the first and second nucleotides as in claim 10; and/or wherein the Tm values of the first and second probes are substantially the same, as in claim 11.

Whitcombe, et al. discloses a method for the detection of diagnostic base sequences in sample nucleic acid, using tailed diagnostic primers having a tag region and a detector region. The method includes contacting a sample under hybridizing conditions and in the presence of appropriate nucleoside triphosphates and an agent for polymerization thereof, with a diagnostic primer for the diagnostic base sequence, the diagnostic primer having a tail sequence comprising a tag region and a detector region, such that an extension product of the diagnostic primer is synthesized when the corresponding diagnostic base sequence is present in the sample, no extension product being synthesized when the corresponding diagnostic base sequence is not present in the sample and any extension product of the diagnostic primer acting as a template for extension of a further primer which hybridizes to a locus at a distance from the diagnostic base sequence; contacting the sample with a tag primer which selectively hybridizes to the complement of the tag sequence in an extension product of the further primer and is extended; and detecting the presence or absence of the diagnostic base sequence by reference to the detector region in the further primer extension product. Note the paragraph bridging pages 1 and 2 of this patent. Note also page 4, lines 22-27, disclosing use of diagnostic and further primers which are genome specific at their 3'-termini but

which carry a detector region and common extensions (tags) at their 5'-termini. Note also from page 6, line 24 through page 7, line 1; and page 10, lines 7-9, of Whitcombe, et al.

It is respectfully submitted that Whitcombe, et al. describes an expression analysis of two or more genes derived from one sample (that is, derived from the same sample). It is respectfully submitted that the method described in Whitcombe, et al. is intended to be used for identifying a very small fraction of a variant sequence in a normal sequence (for example, cancer diagnosis; see, for example, the paragraph bridging pages 6 and 7 of Whitcombe, et al.); or detecting the presence or absence of more than one suspected variant nucleotide in the same sample (note, for example, the first full paragraph on page 7 of Whitcombe, et al.). For example, in the diagnosis of cancer, the expression analysis of Whitcombe, et al. is performed by a two-stage amplification procedure including a first stage to amplify any variant sequence that may be present using an Amplification Refractory Mutation System (ARMS) primer, and a second stage to perform a genomic control reaction in the same reaction vessel using the same primers at low concentrations. Thus, variant sequence(s) and a normal sequence derived from the same sample are identified.

In contrast, and as discussed previously, first and second samples respectively derived from first and second specimens (tissues/organs), e.g., different from each other, are processed. It is respectfully submitted that Whitcombe, et al. would have neither taught nor would have suggested the presently claimed method, and advantages thereof as discussed previously, including highly accurate quantitative analysis of expression levels of targeted genes in first and second samples derived respectively from first and second specimens (in particular, wherein the first and second specimens are different from each other).

Note the following description at page 7, lines 6 and 7, of Whitcombe, et al.:

> "A further and important use of [the Amplification Refractory Mutation System] is for detecting the presence or absence of more than one suspected variant nucleotide in the same sample. [Emphasis added.]"

Thus, it is respectfully submitted that Whitcombe, et al. is clearly concerned with analysis of one sample.

The Examiner has referred to Fig. 17 of Whitcombe, et al., in connection with the presently claimed subject matter, the Examiner asserting that Whitcombe, et al. discloses preparing first nucleotides including a targeted gene by using a first sample (allele A) and preparing second nucleotides including the targeted gene by using a second sample (allele B). It is respectfully submitted, however, that this is an incorrect interpretation of the teachings of Whitcombe, et al., and, in particular, an incorrect interpretation of Fig. 17. It is respectfully submitted that Fig. 17 of Whitcombe, et al. shows single-tube genotyping using primers of the Amplification Refractory Mutation System for each target sequence, that is, allele A and allele B. It is respectfully submitted that allele A and allele B both exist is a single sample derived from the same subject; and it is respectfully submitted that, therefore, Fig. 17 only shows genotyping for each allele derived from one sample.

As set forth previously, it is respectfully submitted that the method according to the present invention differs from the method described in Whitcombe, et al., in that the method of the present invention is a method for gene expression analysis of genes derived from first and second samples respectively from first and second specimens (tissues/organs), e.g., from different specimens (tissues/organs), whereas the method described in Whitcombe, et al. is an expression analysis of

genes derived from one sample (the same sample). Due to this difference, the methods are used for different purposes, as discussed in the foregoing.

Even assuming, arguendo, that the teachings of Shah, et al. were properly combinable with the teachings of Whitcombe, et al., it is respectfully submitted that such combined teachings would have neither disclosed nor would have suggested the presently claimed invention.

Shah, et al. discloses a method for detecting a target nucleic acid fragment directly from a specimen obtained from a patient by in situ hybridization, the method including steps in the listed order of:

(1) Depositing a sample of the specimen onto a slide;

(2) Fixing the sample onto the slide with fixative, the fixative comprising either methanol-acetic acid at a ratio of from 99:1 to 80:20, or formalin-acetic acid at a ratio of from 99:1 to 80:20;

(3) Contacting the nucleic acids of affixed sample with a probe complex specific for the target nucleic acid fragment, under conditions appropriate for hybridization;

(4) Rinsing non-hybridized probe complex from the sample and staining the rinsed sample with Evans Blue; and

(5) Visually detecting the hybridized probe complex by microscopy, with the presence of the probe complex being an indication of the presence of the target nucleic acid fragment.

See column 1, lines 38-54. See also column 2, lines 4-8, 29-33 and 53-56. Note further, column 4, lines 58-63; and column 5, lines 43-47.

Even assuming, arguendo, that the teachings of Shah, et al. were properly combinable with the teachings of Whitcombe, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, the preparation of the first and second nucleotides each including the targeted gene by using first and second samples respectively from first

and second specimens (tissues/organs), as in claims 8 and 14, e.g., which are different from each other, as in claims 13 and 15; and/or the other features of the present invention as discussed previously, and advantages thereof.

Reference by the Examiner to Overbergh, et al. in the Office Action mailed May 16, 2006, is noted. Reliance thereon is improper without including this reference in the statement of the rejection and analyzing this document under the guidelines of 35 USC 103. See In re Hoch, 166 USPQ 406, 407 n.3 (CCPA 1970). It is respectfully submitted that a new rejection in a new Office Action is required, if Overbergh, et al. is being relied upon by the Examiner.

The contention by the Examiner on page 6, lines 1-4, of the Office Action mailed May 16, 2006, that the phrase "for gene expression analysis of genes derived from different samples" is an intended use and does not incorporate a patentably distinguishable feature, is noted. Claim 8 has been amended to positively recite first and second samples being derived respectively from first and second specimens, claim 14 having corresponding language in connection with tissues/organs, these recitations being set forth in connection with active processing steps. It is respectfully submitted that this contention by the Examiner, in connection with intended use, is moot.

The contention by the Examiner on page 6, lines 8-12, of the Office Action mailed May 16, 2006, is respectively traversed, particularly insofar as applicable to the present claims. Thus, the present claims recite, as part of the active processing steps, first and second samples respectively derived from first and second specimens (tissues/organs). Note especially claims 13 and 15. Again, it is emphasized that Whitcombe, et al. expressly states use of a same sample. Giving the present claims their broadest reasonable interpretation, it is respectfully

submitted that Whitcombe, et al. would have neither taught nor would have suggested this aspect of the present invention, and advantages achieved thereby, as discussed previously, wherein highly accurate quantitative analysis of expression levels of targeted genes in first and second samples is achieved.

In view of the foregoing comments and amendments, reconsideration and allowance of all claims presently pending in the above-identified application are respectfully requested.

Applicants request any shortage of fees due in connection with the filing of this paper be charged to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Deposit Account No. 01-2135 (case 1021.43085X00), and credit any excess payment of fees to such Deposit Account.

Respectfully submitted,

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